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Ethanol inhibits the JAK-STAT signaling pathway in freshly isolated rat hepatocytes but not in cultured hepatocytes or HepG2 cells: evidence for a lack of involvement of ethanol metabolism☆

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Abstract

Objectives: To understand the molecular mechanism underlying alcoholic liver injury, effects of acute ethanol on the Janus kinase-signal transducer and activator transcription factor (JAK-STAT) signaling in hepatic cells were studied.
Designs and methods: Effects of acute ethanol on the JAK-STAT signaling in freshly isolated, cultured rat hepatocytes, and HepG2 cells were explored.
Results: Acute ethanol exposure inhibited IL-6- or IFN-activated STAT in freshly isolated hepatocytes but not in cultured hepatocytes, HepG2 cells, or HepG2 cells transfected with alcohol dehydrogenase (ADH) or cytochrome P450(2E1). The inhibitory action of ethanol in freshly isolated hepatocytes was not antagonized by the ADH inhibitor 4-methylpyrazole (4-MP). Acute exposure of hepatocytes to acetaldehyde or hydrogen peroxide did not suppress STAT activation. Further studies indicated that the loss of response to the inhibitory effect of ethanol was not due to hepatocyte proliferation and collagen contact.
Conclusions: Freshly isolated hepatocytes are more susceptible to the inhibitory action of ethanol on the JAK-STAT signaling than cultured hepatocytes or HepG2 cells, which may be implicated in pathogenesis and progression of alcoholic liver disease.

Keywords: Ethanol; Acetaldehyde; 4-Methlepyrazole; Interleukin 6; Interferon; Janus kinase-signal transducer and activator transcription factor (JAK-STAT); Liver; Hydrogen peroxide

1. Introduction

The Janus kinase-signal transducer and activator transcription factor (JAK-STAT) signaling pathway is activated by many cytokines and growth factors and has been implicated in a variety of cellular functions in hematopoietic, immune, neuronal and hepatic system [1–5].[1] In general, the ligation of cytokines to their receptors induces homodimerization of the receptors, which leads to activation of the receptor-associated tyrosine kinases, known as JAKs. This receptor-kinase complex then interacts with and activates SH2-containing cytoplasmic STAT transcription factors, which then translocate to the nucleus to activate the transcription of many genes [1–5]. In the liver, it has been shown that the JAK-STAT signaling pathway activated by interleukin-6 (IL-6), interferons (IFN) and growth hormone plays an important role in hepatic regeneration [6,7], differentiation [8], antiviral and antitumor activities [9,10], acute phase response [11,12] and sexual dimorphism of hepatic gene expression [13]. For example, the essential roles of IL-6-activated STAT3 in liver regeneration and IFN-acti-

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[1] Abbreviations used are: JAK, Janus kinase; STAT, signal transducer and activator transcription factor; IL-6, interleukin-6; IFN-γ, interferon γ; ETOH, ethanol; 4-MP, 4-methylpyrazole; DMSA, DNA mobility shift assay; ADH, alcohol dehydrogenase; CYP2E1, cytochrome p450(2E1); EGF, epidermal growth factor; H2O2, hydrogen peroxide.

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vated STAT1 in antiviral activity were clearly demonstrated in IL-6-deficient and STAT1-deficient mice, respectively [6,7,9,10]. In IL-6-knock out mice, liver regeneration induced by partial hepatectomy was impaired and STAT3 signaling was absent [6,7]. Mice with targeted disruption of the STAT1 gene were more susceptible to viral or bacterial infection and IFN signaling was defective [9,10].

Alcohol is a major etiologic factor of liver disease in Western countries. Chronic excessive consumption of alcohol can lead to steatosis (fatty liver), alcoholic hepatitis, fibrosis, and cirrhosis [14,15], and is associated with high incidence of viral hepatitis and hepatic carcinoma [16,17]. Alcoholic liver disease is a direct result of alcohol-induced hepatotoxicity coupled with impaired hepatic regenerative activity [18–23]. Direct liver injury by ethanol is due to several processes with deleterious effects on the liver, including intracellular accumulation of protein and of acetaldehyde, microsomal activation of hepatotoxins, alterations in hepatic redox state, and enhancement of lymphocyte cytotoxicity [18,19]. An additional mechanism is ethanol-induced impairment of liver regeneration [20–23], which further potentiates and prolongs the ethanol-induced direct liver injury. Our previous data have demonstrated that *in vitro* treatment of freshly isolated hepatocytes with biologically relevant concentrations of ethanol significantly suppressed IL-6-induced STAT3 activation [24,25] and IFN-induced STAT1 activation [26]. Ethanol inhibition of IL-6-activated STAT3 may account for antiregenerative activity of ethanol [21–23]. Negative regulation of IFN-activated STAT1 by ethanol may contribute to the high incidence of viral hepatitis and hepatocellular carcinoma, and the resistance to interferon therapy in alcoholic individuals [16,17, 27–32]. In the present study, we examined the effects of ethanol on STAT activation induced by IL-6 or IFN-γ in cultured hepatocytes and HepG2 cells. Interestingly, we found that up to 200 mM ethanol was unable to suppress IL-6- or IFN-γ-induced STAT activation in cultured hepatocytes or HepG2 cells. Further evidence indicated that ethanol inhibition of IL-6- and IFNγ-induced STAT activation did not require ethanol metabolism and cell proliferating status was not involved.

2. Methods

2.1. Materials

Male Sprague-Dawley rats weighing between 200 to 250 g were purchased from Charles River Laboratories. The human hepatocellular carcinoma HepG2 cell line was obtained from the American Type Culture Collection (Rockville, MD). The following reagents were purchased from Sigma Chemicals (St. Louis, MO): ethanol, acetaldehyde, hydrogen peroxide, collagenase, 4-methylpyrazole (4 MP), IL-6, IFN-γ. Radiolabelled [γ-^{32}P] ATP was from NEN (Boston, MA, USA).

2.2. Transfected HepG2 cells with ADH or CYP2E1

HepG2 cells were transfected stably with ADH or CYP2E1 cDNA as described previously [33,34]. It was tested that ADH and CYP2E1 transfected HepG2 cells were able to metabolize ethanol efficiently in the cytosol and the microsomes, respectively.

2.3. Cell cultures

The HepG2 cells were cultured under conditions specified by the supplier. For experiments, the growth medium was changed to serum-free medium overnight. The cells were then treated with ethanol at various concentrations for 30 min and stimulated with cytokines.

2.4. Isolation and treatment of hepatocytes

The hepatocytes were isolated as described previously [35]. The isolated cells were washed twice and resuspended with Ca^{2+} plus Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, $NaHCO_3$ and 10 mM glucose) containing 1.5% gelatin, and further treated with ethanol, IL-6, or IFNγ. For cell cultures, the isolated cells were washed twice with hepatocyte medium (Dulbecco's modified Eagle's medium containing 1×10^{-8} M dexamethasone, 2.5 μg/mL fungizone, 50 μg/mL gentamycin, 67 μg/mL penicillin, 100 μg/mL streptomycin), and plated onto rat tail collagen-coated culture dishes in hepatocyte medium containing 5% fetal bovine serum. After 2 h, the medium was changed to hepatocyte medium containing 0.5% serum. After 24 h, the cells were treated with buffer, ethanol or acetaldehyde for 30 min then stimulated with IL-6 or IFN-γ. The concentrations for IL-6 and IFN-γ used in these experiments were 20 ng/mL and 5 ng/mL, respectively.

2.5. DNA gel mobility shift assay

DNA gel mobility shift assay for STAT binding was performed as described previously [24–26]. The STAT binding site of oligo m67 (a high affinity serum induce element [SIE] m67) (5′ GTG CAT TTC CCG TAA ATC TTG TCT ACA3′) was used as a probe. In each figure, an autoradiogram representative of at least three independent experiments is shown.

2.6. Partial hepatectomy

Adult male Sprague-Dawley rats were subjected to two thirds partial hepatectomy as described previously [36]. The median and left lateral lobes of the liver were excised. The remnant liver was subjected to collagenase-perfusion [37] 24 h later. The isolated hepatocytes were washed twice and resuspended with Ca^{2+} plus Krebs-Henseleit solution, followed by ethanol and cytokine treatment.

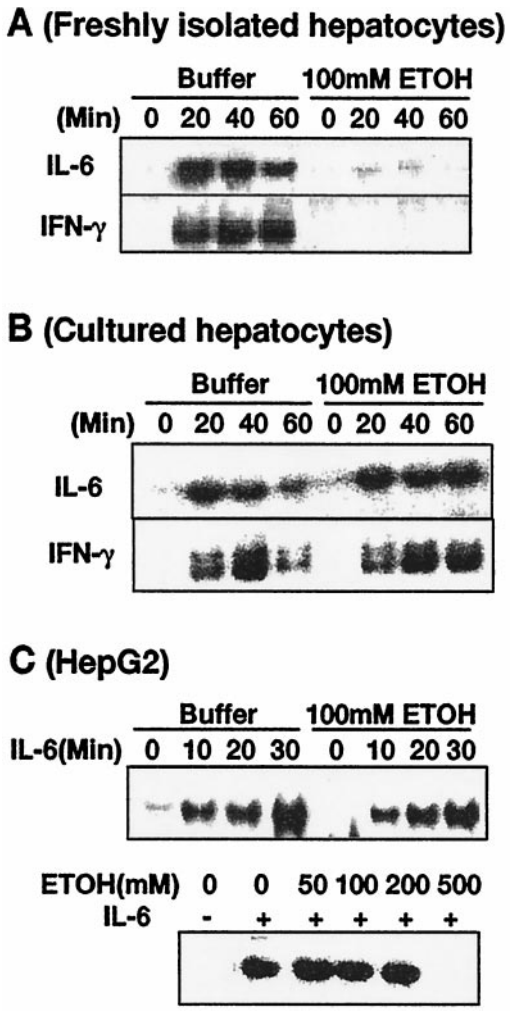

Fig. 1. Acute ethanol exposure inhibits STAT activation in freshly isolated rat hepatocytes but not in cultured hepatocytes or HepG2 cells. Freshly isolated (A), 24 h-cultured adult rat hepatocytes (B) or HepG2 cells (C) were incubated with or without 100 mM ethanol for 30 min, then stimulated with IL-6 or IFN-γ for the indicated times. In panel C, HepG2 cells were also incubated with various concentrations of ethanol for 30 min, followed by a 30-min stimulation with IL-6. STAT activation was determined by DMSA as described under "Materials and Methods."

3. Results

3.1. Acute ethanol treatment inhibits IL-6- or IFN-γ-induced stat activation in freshly isolated rat hepatocytes but not in cultured hepatocytes or HEPG2 cells

For freshly isolated hepatocytes, hepatocytes were used within 30 min after isolation. Freshly isolated hepatoytes were resuspended in Ca^{2+} plus Krebs-Henseleit solution as described under "Materials and Methods". As shown in Figure 1A, IL-6 and IFN-γ rapidly induced STAT3 and STAT1 activation, respectively, and pretreatment with 100 mM ethanol almost completely abolished such activation. To examine the effects of ethanol on cytokine-induced STAT activation in cultured rat hepatocytes, hepatocytes were cultured for 24 h, followed by treating with 100 mM ethanol for 30 min and then stimulating with cytokines. As shown in Figure 1B, IL-6 or IFN-γ rapidly induced STAT activation in cultured hepatocytes and pretreatment with 100 mM ethanol did not suppress such activation, suggesting that culturing hepatocytes for 24 h caused a loss in response to ethanol inhibitory effect on STAT activation. More experiments indicated that culturing hepatocytes for even less than 6 h caused a loss in response to ethanol inhibitory effect (data not shown).

Effects of ethanol on STAT activation were also tested in human hepatocellular carcinoma HepG2 cells. Figure 1C showed that stimulation with IL-6 rapidly induced STAT activation in HepG2 cells, and treatment of HepG2 cells with up to 200 mM ethanol for 30 min did not suppress such activation. These data indicated that acute ethanol exposure did not inhibit STAT activation in cultured hepatocytes or HepG2 cells.

To determine whether the loss in response to the inhibitory effect of ethanol in cultured hepatocytes or HepG2 cells is due to the difference of culture medium, cultured hepatocytes or HepG2 cells were resuspended in the Ca^{2+} plus Krebs-Henseleit solution as used in suspension of freshly isolated hepatocytes. The results demonstrated that up to 200 mM ethanol treatment did not affect STAT activation in cultured hepatocytes or HepG2 cells resuspended in the Ca^{2+} plus Krebs-Henseleit solution (data not shown), suggesting that the loss in response to the inhibitory effect of ethanol in cultured hepatocytes or HepG2 cells is not due to the difference of culture medium.

3.2. Evidence for a lack of involvement of ethanol metabolism in ethanol inhibition of IL-6- or IFNγ-induced STAT activation

The above data demonstrated that acute ethanol exposure inhibited STAT activation in freshly isolated adult rat hepatocytes but not in cultured hepatocytes or HepG2 cells. Since the freshly isolated hepatocytes have the highest ADH activity and the cultured hepatocytes or HepG2 cells have very low ADH activity [20], we wondered if ethanol inhibition of STAT activation might require ethanol metabolism. To test this hypothesis, we performed experiments with 4 methylpyrazole (4-MP), an ADH inhibitor. As shown in Figure 2, 4-MP alone did not affect IL-6-activated STAT (Figure. 2A) and pretreatment of freshly isolated hepatocytes with 0.1 to 1 mM 4-MP did not antagonize the inhibitory effect of ethanol on IL-6- or IFNγ-activated STAT (Figure. 2B). These findings suggest that ethanol inhibition of STAT activation in freshly isolated hepatocytes does not require ethanol metabolism.

To further rule out the involvement of ethanol metabolism in ethanol inhibition of STAT activation, two HepG2 cell lines stably transfected with alcohol dehydrogenase (ADH) [33] or cytochrome p450 (CYP2E1) [34], respectively, were used. These cell lines overexpress ADH or

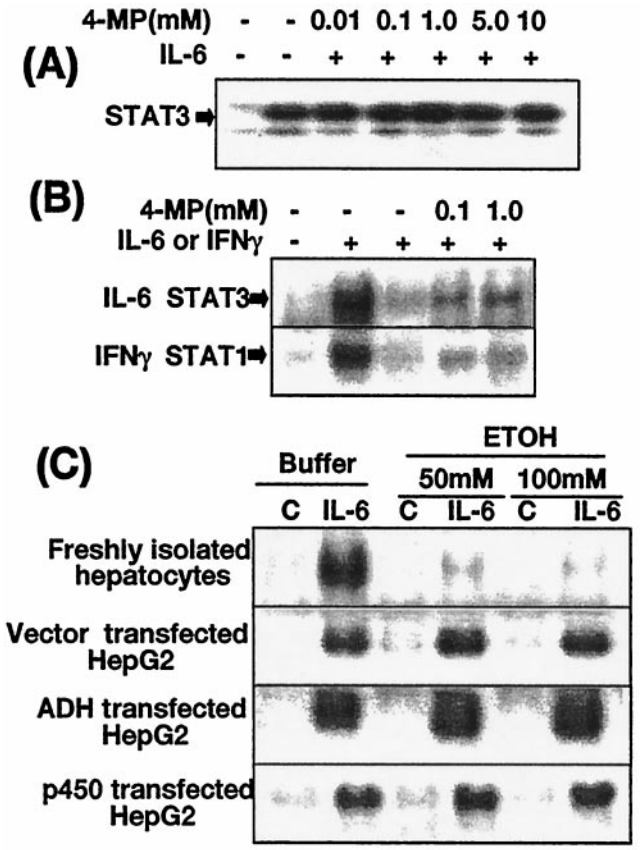

Fig. 2. Evidence for a lack of involvement of ethanol metabolism in ethanol inhibition of STAT activation. (A) Freshly isolated rat hepatocytes were treated with various concentrations of 4-methylprazole (4-MP) for 30 min, followed by a 30-min stimulation with IL-6. (B) Freshly isolated rat hepatocytes were treated with various concentrations of 4-MP for 30 min and then incubated with 100 mM ethanol for 30 min, followed by a 30-min stimulation with IL-6 or IFN-γ. (C) Freshly isolated rat hepatocytes, or HepG2 cells transfected with empty vector, ADH cDNA or CYP2E1 cDNA were incubated with or without 50 to 100 mM ethanol for 30 min, followed by a 30-min stimulation with IL-6. STAT activation was determined by using DMSA.

CYP2E1 enzymes and have been reported to metabolize ethanol efficiently in the cytosol and microsomes, respectively [33,34]. As shown in Figure 2C, ethanol did not attenuate IL-6-induced STAT3 activation in HepG2 cells stably transfected with vector control, ADH, or CYP2E1. These data indicated that the unresponsiveness of HepG2 cells to ethanol inhibition of STAT activation is not due to low levels of ADH or CYP2E1 activity.

3.3. Acetaldehyde, the main ethanol metabolite, did not inhibit IL-6- or IFN-γ-induced STAT activation

Acetaldehyde is the main metabolite responsible for many of the hepatoxic effects of ethanol and has been shown to modulate the activity of several important signaling molecules [38–40]. To determine if acetaldehyde was involved in the ethanol suppression of STAT activation, freshly isolated hepatocytes, cultured hepatocytes, or

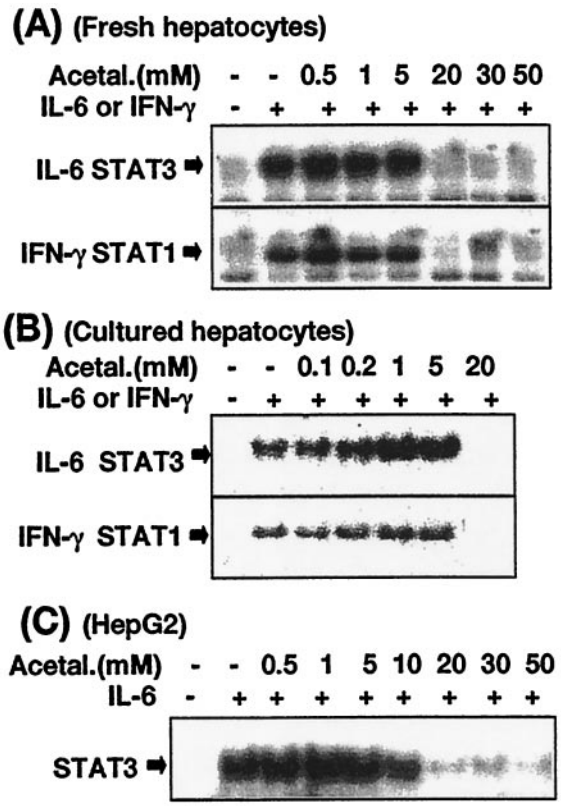

Fig. 3. Effects of acetaldehyde (Acetal.) on IL-6- or IFN-γ-activated STAT in freshly isolated, cultured rat hepatocytes or HepG2 cells. Freshly isolated hepatocytes (A), 24 h-cultured rat hepatocytes (B) or HepG2 cells (C) were incubated with or without various concentrations of acetaldehyde for 30 min, followed by a 30-min stimulation with IL-6 or IFN-γ. STAT activation was determined by DMSA.

HepG2 cells were pretreated with various concentrations of acetaldehyde, followed by stimulation with IL-6 or IFN-γ. As shown in Figure 3, up to 5 mM acetaldehyde did not significantly affect IL-6- or IFN-γ-induced STAT activation in freshly isolated hepatocytes (Figure. 3A), cultured hepatocytes (Figure. 3B), or HepG2 cells (Figure. 3C). Acetaldehyde suppression of STAT activation was only observed at very high toxic concentration (more than 20 mM). These findings suggest that acetaldehyde is not involved in ethanol suppression of STAT activation.

3.4. Hydrogen peroxide potentiates IL-6-induced STAT3 activation in both freshly isolated hepatocytes and cultured hepatocytes

Ethanol oxidation is accompanied by generation of free radicals that may produce cell injury by interacting with different cellular targets such as proteins, DNA, and membrane polyunsaturated fatty acid. It has been shown that free radicals affected the activity of PKC and MAP kinase [41–44]. To check whether generation of free radicals is involved in ethanol inhibition of IL-6-induced STAT activation, freshly isolated hepatocytes or cultured hepatocytes

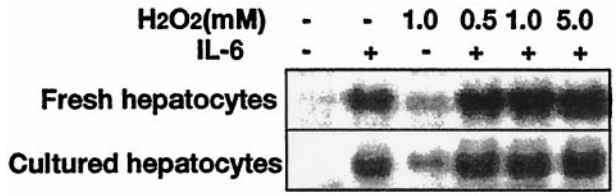

Fig. 4. Effect of hydrogen peroxide on IL-6-induced STAT activation in freshly isolated or cultured hepatocytes. Freshly isolated or 24 h-cultured rat hepatocytes were incubated with or without various concentrations of H2O2 for 15 min, followed by a 30-min stimulation with IL-6. Whole cell extracts were prepared and STAT activation was determined by DMSA.

were pretreated with various concentrations of hydrogen peroxide, followed by stimulation with IL-6. As shown in Figure 4, H2O2 alone slightly activated STAT3 (*lane* 3 vs. *lane* 1), which is consistent with other reports [45,46]. Moreover, 0.5 to 5.0 mM H2O2 slightly potentiated IL-6-induced STAT3 activation in both freshly isolated hepatocytes and cultured hepatocytes, suggesting that generation of free radicals is not involved in ethanol inhibition of IL-6-induced STAT activation.

3.5. Hepatocyte proliferating status is not involved in unresponsiveness to ethanol inhibition of IL-6-induced STAT3 activation in cultured hepatocytes

Unlike freshly isolated hepatocytes, cultured hepatocytes and HepG2 cells are proliferating cells and proliferation may be responsible for the unresponsiveness to ethanol inhibition of IL-6-induced STAT3 activation in cultured hepatocytes or HepG2 cells. To test this possibility, freshly isolated hepatocytes were cultured for 3 h with or without serum or EGF, then incubated with ethanol for 30 min, followed by stimulation with IL-6. As shown in Figure 5A, 100 mM ethanol inhibited IL-6-induced STAT3 activation in both control and cells stimulated with serum or EGF, suggesting that hepatocyte proliferation was not involved in the loss of the ethanol inhibitory effect.

To further rule out the involvement of hepatocyte proliferation in the unresponsiveness of ethanol inhibition of STAT activation in cultured hepatocytes, we examined the effect of ethanol on proliferating hepatocytes prepared from partial hepatectomized rat liver. We chose 24-h partial hepatectomy since proliferation of hepatocytes reached a peak at this time point [47,48]. Hepatocytes were prepared from remnant liver of partial hepatectomized rat liver 24 h after surgery and subjected to ethanol treatment and IL-6 stimulation. As shown in Figure 5B, acute ethanol exposure (50–100 mM) still markedly suppressed IL-6-induced STAT3 activation in hepatocytes isolated from partial hepatectomized rat livers (Figure. 5B), suggesting that proliferation does not affect the inhibitory action of ethanol on IL-6-induced STAT3 activation in cultured hepatocytes.

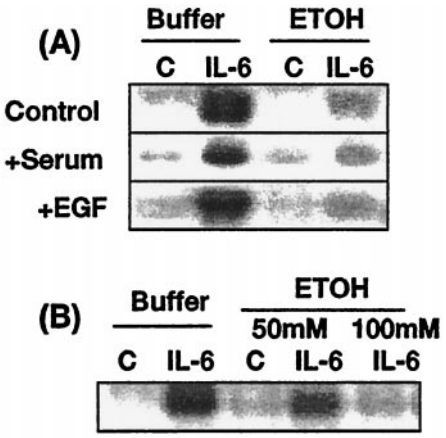

Fig. 5. Effect of hepatocyte proliferation on ethanol inhibition of IL-6-induced STAT3 activation. (A). Freshly isolated hepatocytes were cultured with or without EGF (5 ng/mL) or 5% serum for 3 h as described under "Materials and Methods", then incubated with 100 mM ethanol for 30 min, followed by a 30-min stimulation with IL-6. (B). Hepatocytes isolated from the remnant rat liver after 24 h partial hepatectomy as described under "Materials and Methods" were incubated with 100 mM ethanol for 30 min, followed by a 30-min stimulation with IL-6. Whole cell extracts were prepared and STAT3 activation was determined by DMSA.

3.6. Collagen contact is not involved in unresponsiveness to the inhibitory effect of ethanol on stat activation in cultured hepatocytes

Since cultured hepatocytes were plated on collagen-coated plates, we wondered whether the unresponsiveness of cultured hepatocytes to ethanol inhibition of STAT activation was due to collagen contact. To test this possibility, hepatocytes were seeded on collagen-coated or noncoated plates for 8 h, followed by treating with or without 100 mM ethanol for 30 min and then stimulating with IL-6 for 30 min. As shown in Figure 6, acute ethanol exposure did not significantly inhibit IL-6-activated STAT3 in either collagen-coated or noncoated plates, suggesting that collagen contact is not involved in unresponsiveness to ethanol inhibition of IL-6-activated STAT3 in cultured hepatocytes.

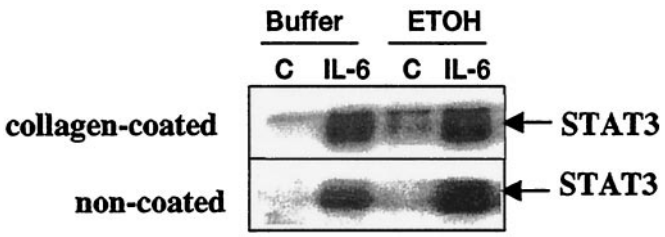

Fig. 6. Collagen contact is not involved in unresponsiveness to ethanol inhibitory effect on STAT activation in cultured hepatocytes. Hepatocytes were plated on collagen-coated or noncoated plates for 8 h, followed by treating with or without 100 mM for 30 min and then stimulating with IL-6 for 30 min. Whole cell extracts were prepared and STAT activation was determined by DMSA.

4. Discussion

The major findings in this paper are that acute ethanol exposure is able to block IL-6- or IFN-γ-induced STAT activation in freshly isolated hepatocytes but not in cultured hepatocytes or HepG2 cells. It was well known that the freshly isolated hepatocytes have high ADH activity while cultured hepatocytes or HepG2 cells have low or no ADH activity [20]. However, five lines of evidence suggest that ethanol metabolism is not required for ethanol inhibition of STAT activation. First, blocking ethanol metabolism with 4-MP, an inhibitor of ADH, did not antagonize ethanol inhibition of STAT activation (Figure. 2). Second, biologically relevant concentrations of acetaldehyde, the major ethanol metabolite, did not suppress STAT activation (Figure. 3). Third, hydrogen peroxide that is produced after ethanol oxidation did not attenuate IL-6-induced STAT activation (Figure. 4). Fourth, acute ethanol also inhibited STAT activation in the proliferating hepatocytes isolated from 24-h partial hepatectomized rat livers (Figure. 5). These hepatocytes have been reported to express low levels of ADH activity [47,49]. Fifth, treatment of freshly isolated hepatocytes with ethanol rapidly (<3 min) inhibited STAT activation [25]. Freshly isolated hepatocytes are quiescent cells, while cultured hepatocytes and HepG2 cells are proliferating cells. However, evidence does not support that proliferating status is involved in ethanol suppression of STAT activation. As shown in Figure 5, EGF or serum treatment did not abolish the ethanol inhibitory effect on STAT activation, and ethanol has a similar inhibitory effect on proliferating hepatocytes isolated from 24-h partial hepatectomized rat livers as on freshly isolated quiescent hepatocytes.

The mechanism by which acute ethanol inhibits STAT activation in freshly isolated hepatocytes but not in cultured hepatocytes or HepG2 cells is not clear. Our previous data showed that activation of protein kinase C is partially (30%) involved in ethanol suppression of STAT activation [26]. Our unpublished data indicated that acute ethanol also stimulated protein kinase C activation in cultured hepatocytes and HepG2 cells. Therefore, the lack of ethanol suppression of STAT activation in cultured hepatocytes and HepG2 cells is not due to absence of activation of protein kinase C. Furthermore, Figure 6 showed that collagen contact is not involved in the unresponsiveness of cultured hepatocytes to ethanol inhibition of STAT activation. It has been reported that many membrane proteins were changed after culturing hepatocytes [50–53], for example, expression of TGFβ receptor [52] and α_{1B} adrenergic receptor [53] markedly decreased after culturing hepatocytes. It is plausible that downregulation of some membrane proteins that are required for ethanol inhibition of STAT activation caused unresponsiveness of cultured hepatocytes or HepG2 cells to ethanol inhibitory effects. Further studies are required to test this hypothesis.

In summary, in present paper we demonstrate that acute ethanol inhibits STAT activation in freshly isolated hepatocytes but not in cultured hepatocytes or HepG2 cells. Although the underlying mechanisms have not been clarified, these findings have already indicated that the JAK-STAT signaling pathway in freshly isolated hepatocytes are more sensitive to ethanol, which may have implications in pathogenesis and progression of alcoholic liver disease.

References

[1] Ihle JN. The Janus protein tyrosine kinase family and its role in cytokine signaling. Adv Immunol 1995;60:1–35.
[2] Liu KD, Gaffen SL, Goldsmith MA. JAK/STAT signaling by cytokine receptors. Curr Opin Immunol 1999;10:271–8.
[3] Hall AK, Rao MS. Cytokines and neurokines: related ligands and related receptors. Trends Neurosci 1992;15:35–7.
[4] Mehler MF, Kessler JA. Hematolymphopoietic and inflammatory cytokines in neural development. Trends Neurosci 1997;20:357–65.
[5] Schindler C. Cytokines and JAK-STAT signaling. Exp Cell Res 1999;253:7–14.
[6] Cressman DE, Diamond RH, Taub R. Rapid activation of the Stat3 transcription complex in liver regeneration. Hepatology 1995;21: 1443–9.
[7] Cressman DE, Greenbaum LE, DeAngelis RA, *et al.* Liver failure, and defective hepatocyte regeneration in interleukin-6-deficient mice. Science.;1996;22:1379–83.
[8] Runge D, Runge DM, Drenning SD, Bowen Jr. WC, Grandis JR, Michalopoulos GK. Growth and differentiation of rat hepatocytes: changes in transcription factors HNF-3, HNF-4, STAT-3, and STAT-5. Biochem Biophy Res Com 1998;250:762–8.
[9] Durbin JE, Hackenmiller R, Simon MC, Levy DE. Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral disease. Cell 1996;4:443–50.
[10] Meraz MA, White JM, Sheehan KC, *et al.* Carver-Moore K, DuBois RN, Clark R, Aguet M, Schreiber RD. Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. Cell 1996;84:431–42.
[11] Schaper F, Gendo C, Eck M, *et al.* Activation of the protein tyrosine phosphatase SHP2 via the interleukin-6 signal transducing receptor protein gp130 requires tyrosine kinase Jak1 and limits acute-phase protein expression. Biochem J 1998;335:557–65.
[12] Richards CD, Brown TJ, Shoyab M, Baumann H, Gauldie J. Recombinant oncostatin M stimulates the production of acute phase proteins in HepG2 cells, and rat primary hepatocytes in vitro. J Immunol.; 1992;148:1731–6.
[13] Udy GB, Towers RP, Snell RG, *et al.* Requirement of STAT5b for sexual dimorphism of body growth rates, and liver gene expression. Proc Natl Acad Sci USA 1997;94:7239–44.
[14] Hill DB, Kugelmas M. Alcoholic liver disease. Treatment strategies for the potentially reversible stages. Postgrad Med 1998;103:261–4.
[15] Weiner FR, Esposti S, Zern M. Ethanol and the liver. In: Arias IM, et al[3rd] Ed. Liver biology and pathobiology. Pp 1383–1412. Raven Press, New. York, 1994.
[16] Brechot C, Nalpas B, Feitelson MA. Interactions between alcohol and hepatitis viruses in the liver. In: Feitelson M, Zern MA, Ed. Clinics in Laboratory Medicine. 16(2), Pp 273–324. Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, 1996.
[17] Farber E. Alcohol and other chemicals in the development of hepatocellular carcinoma. In: Feitelson M, Zern MA, Ed. Clinics in Laboratory Medicine. 16(2), Pp. 377–94. Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, 1996.
[18] Lieber CS. Microsomal ethanol-oxidizing system (MEOS): the first 30 years (1968–1998)–a review;Alcohol Clin Exp Res 1999;23:991–1007.

[19] Arnon R, Esposti SD, Zern MA. Molecular biological aspects of alcohol-induced liver disease. Alcohol Clin Exp Res 1995;19:247–56.
[20] Carter EA, Wands JR. Ethanol-induced inhibition of liver cell function: I. Effect of ethanol on hormone stimulated hepatocyte DNA synthesis and the role of ethanol metabolism. Alochol Clin Exp Res 1988;12:555–62.
[21] Wands JR, Carter EA, Bucher NL, Isselbacher KJ. Inhibition of hepatic regeneration in rats by acute and chronic ethanol intoxication. Gastroenterology 1979;77:528–31.
[22] Diehl AM, Chacon MA, Wagner P. The effect of chronic ethanol feeding on ornithine decarboxylase activity and liver regeneration. Hepatology 1988;8:237–42.
[23] Diehl AM, Thorgeirsson SS, Steer CJ. Ethanol inhibits liver regeneration in rats without reducing transcripts of key protooncogenes. Gastroenterology 1990;99:1105–12.
[24] Chen J, Bao H, Sawyer S, Kunos G, Gao B. Effects of short and long term ethanol on the activation of signal transducer and activator transcription factor 3 in normal and regenerating liver. Biochem Biophy Res Com 1997;239:666–9.
[25] Chen J, Kunos G, Gao B. Ethanol rapidly inhibits IL-6-activated STAT3 and C/EBP mRNA expression in freshly isolated rat hepatocytes. FEBS Lett 1999;457:162–8.
[26] Nguyen VT, Hong F, Ishas EJN, Chen J, Gao B. Interferons activate the p42/44 MAP kinase and JAK-STAT signaling pathways in hepatocytes: differential regulation by acute ethanol via a PKC-dependent mechanism. Biochem J 2000;349:427–34.
[27] Regev A, Jeffers LJ. Hepatitis C, and alcohol. Alcohol Clin Exp Res 1999;23(9):1543–51.
[28] Schiff ER. The alcoholic patient with hepatitis C virus infection. Hepatology 1999;26:39S–42S.
[29] Degos F. Hepatitis C, and alcohol. J Hepatol.;1999;31(Suppl 1): 113–8.
[30] Ohnishi K, Matsuo S, Matsutani K, *et al.* Interferon therapy for chronic hepatitis C in habitual drinkers: comparison with chronic hepatitis C in infrequent drinkers. Am J Gastroenterol 1996;91:1374–9.
[31] Okazaki T, Yoshihara H, Suzuki K, *et al.* Efficacy of interferon therapy in patients with chronic hepatitis C. Comparison between non-drinkers and drinkers. Scand J Gastroenterol 1994;29:1039–43.
[32] Mochida S, Ohnishi K, Matsuo S, Kakihara K, Fujiwara K. Effect of alcohol intake on the efficacy of interferon therapy in patients with chronic hepatitis C as evaluated by multivariate logistic regression analysis. Alcohol Clin Exp Res 1996;20:371–7.
[33] Clemens DL, Halgard CM, Miles RR, Sorrell MF, Tuma DJ. Establishment of a recombinant hepatic cell line stably expressing alcohol dehydrogenase. Arch Biochem Biophys 1995;321:311–8.
[34] Dai Y, Cederbaum AI. Inactivation and degradation of human cytochrome P4502E1 by CCl4 in a transfected HepG2 cell line. J Pharmacol Exp Ther 1995;275:1614–22.
[35] Preiksatis H, Kan WH, Kunos G. Decreased alpha 1-adrenoceptor responsiveness and density in liver cells of thyroidectomized rats. J Biol Chem 1982;257:4321–7.
[36] Gao B, Jiang L, Kunos G. Transcriptional regulation of α1b adrenergic receptors (α1bAR) by nuclear factor 1 (NF1): a decline in the concentration of NF1 correlates with the downregulation of α1bAR gene expression in regenerating liver. Mol Cell Biol 1996;16:5997–6008.
[37] Spector M, Auer K, Jarvis W, *et al.* Differential regulation of the mitogen-activated protein and stress-activated protein kinase cascades by adrenergic agonists in quiescent and regenerating adult rat hepatocytes. Mol Cell Biol 1997;17:3556–65.
[38] Anania FA, Womack L, Potter JJ, Mezey E. Acetaldehyde enhances murine alpha2(I) collagen promoter activity by Ca2+-independent protein kinase C activation in cultured rat hepatic stellate cells. Alcohol Clin Exp Res 1999;23:279–84.
[39] Casini A, Galli G, Salzano R, *et al.* Acetaldehyde induces c-fos, and c-jun proto-oncogenes in fat-storing cell cultures through protein kinase C activation. Alcohol Alcohol 1994;29:303–14.
[40] Koivisto T, Salaspuro M. Acetaldehyde alters proliferation, differentiation and adhesion properties of human colon adenocarcinoma cell line Caco-2. Carcinogenesis 1998;19:2031–6.
[41] Gopalakrishna R, Anderson WR. Ca2+-, and phospholipid-independent activation of protein kinase C by selective oxidative modification of the regulatory domain. Proc Natl Acad Sci USA 1989;86:6785–62.
[42] Gopalakrishna R, Anderson WR. Reversible oxidative activation and inactivation of protein kinase C by the mitogen/tumor promoter periodate. Arch Biochem Biophys 1991;285:382–87.
[43] Aikawa R, Komuro I, Yamazaki T, et al. Oxidative stress activates extracellular signal-regulated kinases through Src and Ras in cultured cardiac myocytes of neonatal rats. J Clin Invest 1997;100:1813–21.
[44] Stevenson MA, Pollock SS, Coleman CN, Calderwood SK. X-irradiation, phorbol esters, and H2O2 stimulate mitogen-activated protein kinase activity in NIH-3T3 cells through the formation of reactive oxygen intermediates. Cancer Res 1994;54:12–5.
[45] Carballo M, Conde M, El Bekay R, *et al.* Oxidative stress triggers STAT3 tyrosine phosphorylation, and nuclear translocation in human lymphocytes. J Biol Chem. 1999;274:17580–6.
[46] Simon AR, Rai U, Fanburg BL, Cochran BH. Activation of the JAK-STAT pathway by reactive oxygen species. Am J Physiol 1998; 275:C1640–52.
[47] Zarnegar R, DeFrances MC, Kost DP, Lindroos P, Michalopoulos GK. Expression of hepatocyte growth factor mRNA in regenerating rat liver after partial hepatectomy. Biochem Biophys Res Commun 1991;177:559–65.
[48] Michalopoulos GK, DeFrances MC. Liver regeneration. Science 1997;276:60–6.
[49] Paso AR, Poso H. Ethanol elimination in regenerating rat liver: the roles of alcohol dehydrogenase and acetaldehyde. Acta Chem Scand [B] 1979;33:249–55.
[50] Sidu JS, Farin FM, Omiecinski CJ. Influence of extracellular matrix overlay on phenobarbital-mediated induction of CYP2B1, 2B2, and 3A1 genes in primary adult rat hepatocyte culture. Arch Biochem Biophys 1993;301:103–13.
[51] Kim TH, Bowen WC, Stolz DB, Runge D, Mars WM, Michalopoulos GK. Differential expression and distribution of focal adhesion and cell adhesion molecules in rat hepatocyte differentiation. Exp Cell Res 1998;244:93–104.
[52] Nishikawa Y, Wang M, Carr BI. Changes in TGF-beta receptors of rat hepatocytes during primary culture and liver regeneration: increased expression of TGF-beta receptors associated with increased sensitivity to TGF-beta-mediated growth inhibition. J Cell Physiol 1998;176:612–23.
[53] Ishac EJ, Lazar-Wesley E, Kunos G. Rapid inverse changes in alpha 1B- and beta 2-adrenergic receptors and gene transcripts in acutely isolated rat liver cells. J Cell Physiol 1992;152:79–86.